

## ***Interactive comment on “Fungi regulate response of N<sub>2</sub>O production to warming and grazing in a Tibetan grassland” by Lei Zhong et al.***

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Received and published: 30 April 2018

Thank you for your suggestions. We have revised our manuscript “Fungi regulate response of N<sub>2</sub>O production to warming and grazing in a Tibetan grassland”, based on your comments. We have carefully addressed each comment and our responses to these comments are listed below. The attachments are the manuscript which had improved as your suggestions. We hope that all necessary revisions have been made. However, we would be prepared to make further revisions and modifications if required.

Responses to the Reviewer's comments:

[Comments] 1. The experimental design is not acceptable. Firstly, why did you choose “winter grazing”? There seems no explanation. The temperature should be too low to

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let the animal graze out of the field in winter. Additionally, the grassland is expected to be covered by snow and the grasses should be withered in winter. Secondly, the description of the treatment is really confusing. Winter grazing should be used in the current study, but “For grazing treatments, the grazing treatments in this site were used for summer grazing treatments until 2010, from 2011 to 2015, there was no grazing during the summer, and grazing was replaced by cutting and removing about 50

[Responses] Sorry, our previous description caused the misunderstanding by the referee. In the new version, we clarified why we used winter grazing. On the Qinghai-Tibet plateau, winter grazing is very common and alpine meadows are generally classified into two grazing seasons, i.e. warm season grazing from June to September and cold season grazing from October to May even the grassland was covered by snow (Cui et al., 2015). Winter pasture contributed about 40

In the new version, we clarified our design. During 2006–2010 summer grazing treatments were used to explore the effects of warming and grazing on ecosystem during the growing seasons (Luo et al. 2010; Hu et al. 2010; Wang et al. 2012). Considering strong disturbance, grazing was stopped during 2011–2015. Given the importance of winter grazing, winter grazing during the non-growing seasons was further investigated (Zhu et al. 2015; Che et al. 2018). We agree with the referee that grazing cannot be simulated by cutting or mowing since grazing involves tread and urine/dung deposition. However, during winter, such effects could be very small because soil and dung are frozen and tread has little effect on soil. Actually, we had examined how clipping simulated the effects of actual grazing before we established four replicated “actual grazing treatments” compared with the “simulated grazing treatments”, the soil and plant all showed no difference between simulated grazing and actual grazing treatments (Klein et al. 2004; 2007), and showed the urine/dung deposition and tread by animals' effect on soil and plant is limited. We believe that removal of litter can stimulate the effect of winter grazing, which has been demonstrated by previous studies (Zhu et al. 2015; Che et al. 2018). We had improved the description of the winter grazing treatment and

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make it more clearly, please see lines 159-174.

[Comments] 2. I can't see how you can jump from nitrification or denitrification potentials to assessing the contributions of bacterial and fungi to potential N<sub>2</sub>O emissions. Nitrification or denitrification potentials should not be regarded as N<sub>2</sub>O productions especially emissions by nitrification or denitrification. From this sense, the discussion section should be rewritten thoroughly.

[Responses] Most studies mainly focused on the contribution of bacterial nitrification and denitrification to potential N<sub>2</sub>O emissions. Because numerous studies have shown that fungal nitrification and denitrification can play an important role in N<sub>2</sub>O production. Therefore, in this study we aimed to quantify the contribution of fungal and bacterial to potentials of N<sub>2</sub>O from nitrification and denitrification. Because the contribution of fungal nitrification and denitrification was higher than bacteria's (Fig. 5) in control treatment, this indicates that the fungi played the major role in potential N<sub>2</sub>O emissions.

We agreed with the referee that the nitrification or denitrification potentials should not be regarded as N<sub>2</sub>O productions especially emissions. In this study, we mainly focused on the mechanism of N<sub>2</sub>O produce process and distinguished the role of bacteria and fungi in N<sub>2</sub>O produce process. In the new version, we rewrote the discussion section and related sections to avoid the misunderstanding.

[Comments] 3. The manuscript is not well prepared. There are lots of writing issues throughout the manuscript. I only presented few of them since there are too many.

[Response] In the new version, we almost rewrote the manuscript and asked a native English speaker Miss Ri Weal to polish the language errors. We hope the new version is easy to read and follow.

[Comments] Abstract Lines 44-46: The treatments should be described briefly in the abstract to increase the readability. Additionally, some key information about the method should be presented.

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[Responses] Done. Please see lines 44-48.

[Comments] Lines 46-52: The values should be presented with uncertainties, e.g., standard error, standard deviation or 95

[Responses] Done. Please see lines 48-49 and Fig.5.

[Comments] Lines 46-47: Were these values got from the control?

[Responses] Yes, these values are obtained from the control. We clarified this in the new version, please see lines 49.

[Comments] Lines 49-52: Suggest rephrase these sentences in such way: "However, warming significantly increased the enzyme activity of bacterial nitrification and denitrification to 53

[Responses] Done. Please see lines 53-56.

[Comments] Lines 52-54: How could you make this conclusion? Under what conditions do soil fungi contribute more to N<sub>2</sub>O production? This sentence is of course not clear. If the conclusion is obtained based on results from the control, it should be put somewhere after lines 46-47. Additionally, can you make such a strong conclusion based on an incubation experiment?

[Responses] Thank the referee for pointing out the question. We rewrote the abstract as the referee suggested, please see lines 40-62. Our conclusion was based on the role of fungi and bacteria in N<sub>2</sub>O produce process by the incubation experiment but not in N<sub>2</sub>O emissions. In the new version, we clarified this, please see lines 1-62.

[Comments] Lines 56-58: This should not be put in the abstract as a key implication since it should be regarded as a fact.

[Responses] Done. Please see lines 58-60.

[Comments] Line 59-60: This sentence should be rephrased since some grammar

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issue exists. For example, “lead to refine: : :.” is not correct. Overall, the abstract needs substantial revision.

[Responses] Done. Please see lines 40-62.

[Comments] Introduction Line 66: not clear what does “it” refer to.

[Responses] “it” refer to N<sub>2</sub>O emission, we clarified it, please see lines 67-69.

[Comments] Lines 67-69: This sentence needs substantial revision.

[Responses] Done, please see lines 69-71.

[Comments] Line 122: Why did you choose “winter grazing”? There seems no explanation. The temperature should be too low to let the animal grazing out of the field in winter. Additionally, the grassland is expected to be covered by snow and the grasses should be withered in winter.

[Responses] Sorry, our previous description caused the misunderstanding by the referee. In the new version, we clarified why we used winter grazing. On the Qinghai-Tibet plateau, winter grazing is very commonly and alpine meadows are generally classified into two grazing seasons, i.e. warm season grazing from June to September and cold season grazing from October to May even the grassland was covered by snow (Cui et al., 2015). Winter pasture contributed about 40

[Comments] M M Lines 130-131: The symbol C is not correctly used.

[Responses] Done, please see lines 136-137.

[Comments] Lines 131-132: over 80

[Responses] Over 80

[Comments] Lines 133-134: Please clearly present the soil classification systems and the references.

[Responses] Done, please see lines 139.

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[Comments] Lines 134: There should be a space between the word and the parentheses here and in other sentences or Figures (Please check the figures as well).

[Responses] Done, please see lines 142 and the caption of figures.

[Comments] Line 139: The indent here is not consistent with other paragraphs. Please keep consistency.

[Responses] Done, please see lines 145.

[Comments] Line 146: delete was.

[Responses] Done, please see lines 152.

[Comments] Lines 153-156: The description is really confusing. According to the above paragraph, winter grazing was used in the current study, but “For grazing treatments, the grazing treatments in this site were used for summer grazing treatments until 2010, from 2011 to 2015, there was no grazing during the summer, and grazing was replaced by cutting and removing about 50

[Responses] In the new version, we clarified our design. During 2006-2010 summer grazing treatments was used to explore the effects of warming and grazing on ecosystem during the growing seasons (Luo et al. 2010; Hu et al. 2010; Wang et al. 2012). Considering strong disturbance, grazing was stopped during 2011-2015. Given the importance of winter grazing, winter grazing during the non-growing seasons was further investigated (Zhu et al. 2015; Che et al. 2018). We agree with the referee that grazing cannot be simulated by cutting or mowing since grazing involves tread and urine/dung deposition. However, during winter, such effects could be very small because soil and dung are frozen and tread has little effect on soil. Actually, we had examined how clipping simulated the effects of actual grazing before we established four replicated “actual grazing treatments” compare with the “simulated grazing treatments”, the soil and plant all showed no difference between simulated grazing and actual grazing treatments (Klein et al. 2004; 2007), and showed the urine/dung deposition and tread by

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animals' effect on soil and plant is limited. We believe that removal of litter can stimulate the effect of winter grazing, which has been demonstrated by previous studies (Zhu et al. 2015; Che et al. 2018). We had improved the description of the winter grazing treatment and make it more clearly, please see lines 159-174.

[Comments] Lines 195-196: Please revise this title.

[Responses] Done, please see lines 204-205.

[Comments] Line 201 and line 235: The monthly mean temperature was 9.7 C in August, but the slurry was incubated under 28 C. The incubation temperature is nearly two times greater than the mean temperature. How would this artificial effect modulate the responses of the measured indices?

[Responses] The incubation experiment was measured the soil ability/potential of N<sub>2</sub>O production, not the field N<sub>2</sub>O flux. The method was provided a good condition for the soil microbial, eg. relative high incubation temperature, and added some substrate, so that can inspire the activities of nitrifying and denitrifying microorganism and show the ability N<sub>2</sub>O produce by nitrifying and denitrifying microorganism in soils. The method and the incubation condition was commonly used to measure the nitrification and denitrification enzyme activity and proved to be useful (Smith and Tiedje, 1979; Simek and Hopkins, 1999; Chroňáková et al. 2009; Cantarel et al. 2012).

[Comments] Line 203: What "them" stands for?

[Responses] "them" stands for slurry, we clarified it. Please see lines 212.

[Comments] Line 220: nitrification again?

[Responses] It is denitrification, we corrected it. Please see lines 230.

[Comments] 3. Results and Discussion Lines 286-291: I can't see how you can jump from nitrification or denitrification potentials to assessing the contributions of bacterial and fungi to potential N<sub>2</sub>O emissions. Nitrification or denitrification potentials should

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not be regarded as N<sub>2</sub>O productions especially emissions by nitrification or denitrification. From this sense, the discussion section should be rewritten thoroughly.

[Responses] Most studies mainly focused on the contribution of bacterial nitrification and denitrification to potential N<sub>2</sub>O emissions. Because numerous studies have shown that fungal nitrification and denitrification can play an important role in N<sub>2</sub>O production. Therefore, in this study we aimed to quantify the contribution of fungal and bacterial to potentials of N<sub>2</sub>O from nitrification and denitrification. Because the contribution of fungal nitrification and denitrification was higher than bacteria's (Fig. 5) in control treatment, this indicates that the fungi played the major role in potential N<sub>2</sub>O emissions.

We agreed with the referee that the nitrification or denitrification potentials should not be regarded as N<sub>2</sub>O productions especially emissions. In this study, we mainly focused on the mechanism of N<sub>2</sub>O produce process and distinguished the role of bacteria and fungi in N<sub>2</sub>O produce process. In the new version, we rewrote the discussion section and related sections to avoid the misunderstanding.

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Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2017-552/bg-2017-552-AC4-supplement.pdf>

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-552>, 2018.